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#### **REMARKS**

The presently claimed invention features probes that recognize certain variant sequences within the MTHFR gene and methods for using such probes.

On October 19, 2001 Applicants filed a Sequence Listing containing a nucleotide sequence of the MTHFR gene and several other genes, all of which were identified by name and GenBank® Accession Number in Table 10 of the present application. The Sequence Listing was used to amend the application to include the actual nucleotide sequences of the genes identified by name and GenBank® Accession Number in Table 10 of the application. The Sequence Listing was accompanied by a Declaration Regarding Incorporation by Reference. This declaration, signed by Vincent P. Stanton, Jr., stated that the sequences in the Sequence Listing were identical to those incorporated by reference in the application by inclusion of the GenBank® Accession Numbers in Table 10.

It has recently come to Applicants' attention that at least the sequence of the MTHFR gene in the October 19, 2001 Sequence Listing is not correct. Accordingly, Applicants have today submitted (sent to Box Sequence, U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA, 22202; a paper copy is enclosed herewith) a replacement sequence listing containing the correct MTHFR nucleotide sequence. Also enclosed is a Declaration Regarding Incorporation by Reference signed by Vincent P. Stanton, Jr. This declaration states that:

Applicant hereby declares that the Sequence Listing appended hereto consists of the same sequence information incorporated by reference in the above-referenced application by reference to the GenBank® Accession Number U09806.

The sequence of SEQ ID NO:1 in the appended Sequence Listing is the same as that associated with GenBank® Accession number U09806 on July 20, 1998, the filing date of U.S. Serial No. 60/093,484, from which the present application claims priority. This particular version of GenBank® Accession No. U09806 is assigned the version identifier GI:945022. Exhibit A attached hereto is a printout from the GenBank® Database of GenBank® Accession No. U09806 [GI: 945022]. This printout shows that GenBank® Accession No. U09806 [GI: 945022] replaced an earlier version of the sequence GenBank® Accession No. U09806 [GI: 945022] on August 17, 1995. Exhibit B is a printout from the

<sup>&</sup>lt;sup>1</sup> The sequences of the other genes identified in Table 10 are not included in the sequence listing because the pending claims concern only the MTHFR sequence.

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GenBank® Database of GenBank® Accession No. U09806 [GI:6174884] showing that it replaced GenBank® Database of Accession No. U09806 [GI:945022] on November 2, 1999.

In my Declaration regarding Incorporation By Reference filed on October 22, 2001 I mistakenly stated that the sequences in the Sequence Listing appended thereto were those incorporated by reference to GenBank® Accession numbers in the above-referenced application. However, for GenBank® Accession number U09806, the Sequence Listing appended to my October 22, 201 Declaration Regarding Incorporation by Reference had the incorrect version of GenBank® Accession number U09806, namely, GenBank® Accession number U09806 [GI:6174884] rather than GenBank® Accession number U09806 [GI:945022]. This error was made without deceptive intent.

Applicants recognize that the previously pending claims have been allowed. However, Applicants request that the Examiner examine the pending claims based on the corrected MTHFR nucleotide sequence in the Sequence Listing submitted herewith.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a \$370.00 check for the Request for Continued Examination fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully symmitted,

Date: 13 NOU 2002

Anita I. Meiklejohn, Ph D

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# Version with markings to show changes made

## In the specification:

Table 10 beginning at page 171 has been amended as follows:

### Table 10

### Variance Table

Hugo V	GID ariance Start	OMIM ID Variance	VGX	Symbol	Description
U73338	U73338	156570	GEN-	-69	Methionine
Synthase [(	SEQ ID NO:1)]				
	194	(-201)C>C	3	5	ſ
	284	(-111)C>T	ľ	5	1
	1136	742G>F	4	V2481	M.
	1252	858C>1	[	Silent	_
	1334	940G>A	A.	D3141	V
	1699	1305T>C	2	Silent	<u>.</u>
	3150	2756A>0	3	D9190	3
	3207	2813G>1	<u>.</u>	S9381	Ţ
	3209	2815G>0	2	G939F	२
	5444	5050C>F	A	3 '	1
	5551	5157G>F	4	3 '	1
	5573	5179C>T		3 '	T .
	5659	5265T>C		3 '	1
	5678	5284T>C	;	3'	1
	5874	5480C>T		3 '	1
	5934	5540A>0	3	3 '	ı
D78586	D78586	114010	GEN-	·BR	CAD PROTEIN
[(SEQ ID NO	:2)]				
	3434	3408C>T		Silent	-
	4313	4287T>C	:	Silent	•
	4799	4773A>0	;	Silent	- •
	5255	5229C>T	1	Silent	-
	5455	5429G>A	٠ '	R18100	)
	5507	5481T>C	:	Silent	:
	5810	5784C>T	•	Silent	•
	6128	6102C>T	1	Silent	
	6626	6600C>T	1	Silent	
	6686	6660C>T	1	Silent	:
U09178	U09178	274270	GEN-	HA	
Dihydropyrin	midine Dehydro	genase [(SEQ	ID	NO:3)]	
	166	85T>C		C29R	}

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577 M166V 496A>G 638 Y186C 557A>G 1708 1627A>G I543V 3' 3432 3351T>C 3' 3682 3601C>T 3730 3649G>A 3 ' 3925 3844A>G 3' 31 3937 3856T>C U19720 U19720 600424 GEN-I1 Folate Transporter (SLC19A1) [(SEQ ID NO:4)] 175 R27H 80G>A 341 246C>G Silent 791 Silent 696C>T 1067 Silent 972G>A 1337 1242C>A Silent 3' 1997 1902T>C 31 2005^2006insG 2100 2582 2487T>G 3 ' 2522C>T 3 ' 2617 3' 2652 2557T>C Homo sapiens reduced U92868 U92868 600424 GEN-LUK folate carrier (RFC1) gene, exons 1a, 1c and 1b [(SEQ ID NO:5)] 431 431A>G Intron 441 441A>G Intron 498 498C>T Intron 579 579G>C Intron 599 599G>C Intron 188350 GEN-KL X02308 X02308 Thymidylate synthetase [(SEQ ID NO:6)] 3 ' 1066 961T>C 31 1136 1031A>G 1497 1392T>A 3 ' D00517 D00517 188350 GEN-LUC Thymidylate synthase, promoter [(SEQ ID NO:7)] 276 276C>T Intron 321 321T>C Intron 452 452G>A Intron 457 457^insC Intron 491 491C>A Intron 533 533T>C Intron 624 624A>C Intron 639 639A>G Intron 655T>C 655 Intron

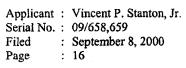
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gene for thymidylate synthase, exons 1, 2, 3, 4, 5, 6, 7, complete cds [(SEQ ID NO:8)]

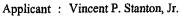
ds [(SEQ	ID NO:8)]			
701		701A>C	Intron	
716		716A>G	Intron	
732		732T>C	Intron	
1293		1293A>G	Intron	
1322		1322C>G	Intron	
1379		1379T>C	Intron	
1590		1590C>T	Intron	
1688		1688C>G	Intron	
2401		2401A>G	Intron	
2429		2429G>A		
2488		2488C>T		
2594		2594G>T		
2618		2618G>A	Intron	
3083		3083G>A	Intron	
3125		3125G>A	Intron	
3212		3212C>T	Intron	
3619		3619T>A	Intron	
3635		3635G>A	Intron	
4256		4256G>A	Intron	
4898		4898A>G	Intron	
5006		5006C>T	Intron	
5062		5062G>A	Intron	
5167		5167G>A	Intron	
11069		11069A>G		
11238		11238C>T		
11293		11293T>G	Intron	
11422		11422T>C	Intron	
11686		11686C>T	Intron	
12598		12598T>C	Intron	
13171		13171T>C	Intron	
13298		13298G>A	Intron	
13645		13645T>C	Intron	
13751		13751C>A	Intron	
13782		13782T>C	Intron	
13806		13806T>C	Intron	
13813		13813T>C	Intron	
14479		14479A>G	Intron	
14546	14	546^insT	Intron	
14585		14585C>T	Intron	
14729		14729G>A	Intron	
14787		14787C>T	Intron	
14795		14795G>A	Intron	



15041	15041T>C	Intron	
15343	15343G>A	Intron	
15449	15449G>A	Intron	
15502	15502G>A	Intron	
15545	15545C>T	Intron	
15589	15589A>G	Intron	
15769	15769C>T	3'	
15839	15839A>G	3'	
16148	16148G>A	3'	
16198	16198T>G	3'	
16202	16202G>T	Intron	
X59618 X59618		M3 Ribonucleotide	
		45 Albonucleotide	
reductase M2 polypepti 128		5'	
	(-67)G>A	5 <b>'</b>	
189	(-6) T>G		
524	330C>G	Silent	
1399	1205T>A	3'	
1464	1270G>A	31	
1636	1442C>T	3'	
1738	1544C>T	3'	
2259	2065T>C	3'	
S72487 S72487	131222 GEN-31	LD Thymidine	
phosphorylase, partial			
183	19G>A	D7N	
483	319C>T	3'	
601	437G>C	3 *	
1299	1135G>A	3'	
M58602 M58602	131222 GEN-LUB	Thymidine	
phosphorylase, promote			
124	124C>T	3'	
439	439G>A	3'	
1044	1044^insCT	3'	
1331	1331G>A	3'	
1977	1977G>A	Intron	
2149	2149G>A	Intron	
2467	2467A>G	Intron	
2634	2634C>G	Intron	
2975	2975G>A	Intron	
3116	3116G>T	Intron	
3255	3255A>C	Intron	
3344	3344T>C	Intron	
4051	4051C>A	Intron	
4782	4782G>A	Intron	
5022	5022T>C	Intron	
5266	5266G>A	Intron	

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5285	5285C>G	Intron
5438	5438T>A	Intron
5482	5482C>T	Intron
5629	5629G>A	Intron
5648	5648C>T	Intron
5731	5731G>A	Intron
M98045 M98045	136510 GEN-	4C3 Homo sapiens
folylpolyglutamate synthe		<del>-</del>
NO:12)]	•	
802	732C>T	Silent
1747	1677G>T	3'
1900	1830T>C	3'
	510 GEN-LUE	Human
folylpolyglutamate synthe	tase (FPGS) gene	e, exons 5-11, and
partial cds [(SEQ ID NO:1		·
1424	1424C>A	Intron
1649	1649G>A	Intron
2554	2554A>G	Intron
U24252 U24252	136510 GEN-1	LUF
Folylpolyglutamate synthe	tase, promoter a	and exons 1-4 [(SEQ ID
NO:14)]	· •	
263	263A>G	Intron
266	266G>T	Intron
527	527C>G	Intron
1037	1037A>G	5'
1139	1139G>A	Intron
1217	1217C>T	Intron
1647	1647C>T	Intron
1955	1955G>A	Intron
2017	2017G>A	Intron
2037	2037G>A	Intron
2189	2189A>G	Intron
2282	2282C>T	Intron
2309	2309A>G	Intron
U09806 U09806	236250 GEN-	4FZ Human
methylenetetrahydrofolate	reductase mRNA	partial cds [(SEQ ID
NO:15)] (SEQ ID NO:1)		-
120	120T>C	Silent
464	464T>G	M155R
519	519C>T	Silent
668	668C>T	A223V
1059	1059T>C	Silent
1289	1289C>A	3'
1308	1308T>C	3'
1784	1784G>A	3'



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AF061655 deaminase,	AF061655		3920 ID NO	GEN-LU:16)1	J Cytidine
acamirmaco,	575	[ (===		575T>C	Intron
	648			648T>C	Intron
	771			771G>C	Intron
	883			883G>A	Intron
	941		94	1^insC	5'
	1051		1	051A>C	K27Q

### In the claims:

or the complement thereof.

Claims 182-201 have been amended as follows:

182. An isolated nucleic acid probe comprising at least 15 contiguous nucleotides of the nucleotide sequence of [SEQ ID NO:15] <u>SEQ ID NO:1</u> (methylenetetrahydrofolate reductase), the probe comprising at least one of:

(a)	nucleotide 120 of SEO ID NO:1 wherein T is replaced by C;
<u>(b)</u> [(a)]	nucleotide 464 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N] T is
replaced by G;	
(c) [(b)]	nucleotide 519 of [SEQ ID NO:15] $\underline{SEQ\ ID\ NO:1}$ wherein [N] $\underline{C}$ is
replaced by T;	
<u>(d)</u> [(c)]	nucleotide 668 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N] C is
replaced by T;	
(e) [(d)]	nucleotide 1059 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N] $\underline{T}$ is
replaced by C;	
<u>(f)</u> [(e)]	nucleotide 1289 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>C</u> is <u>is</u>
replaced by A;	
(g) [(f)]	nucleotide 1308 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N] $\underline{T}$ is
replaced by C; and	
<u>(h)</u> [(g)]	nucleotide 1784 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N] G is
replaced by A;	<b>,</b>

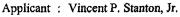
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T;

- 183. An isolated nucleic acid probe comprising at least 15 contiguous nucleotides of the nucleotide sequence of [SEQ ID NO:15] <u>SEQ ID NO:1</u> (methylenetetrahydrofolate reductase), the probe comprising at least two of:
  - (a) nucleotide 120 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u> is <u>replaced by C</u>;
- (b) nucleotide 464 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u> is <u>replaced by</u> G;
  - (c) nucleotide 519 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>C</u> is <u>replaced by T</u>;
  - (d) nucleotide 668 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>C</u> is <u>replaced by</u>
- (e) nucleotide 1059 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u> is <u>replaced by</u> C;
- (f) nucleotide 1289 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>C</u> is <u>replaced by</u> A;
- (g) nucleotide 1308 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u> is <u>replaced by</u> C; and
- (h) nucleotide 1784 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>G</u> is <u>replaced by</u> A; or the complement thereof.
- 184. The probe of claim 182 or 183 comprising no more than 500 contiguous nucleotides of [SEQ ID NO:15] <u>SEQ ID NO:1</u>.
- 185. The probe of claim 182 or 183 comprising no more than 200 contiguous nucleotides of [SEQ ID NO:15] <u>SEQ ID NO:1</u>.
- 186. The probe of claim 182 or 183 comprising no more than 100 contiguous nucleotides of [SEQ ID NO:15] <u>SEQ ID NO:1</u>.



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187. The probe of claim 182 or 183 comprising no more than 50 contiguous nucleotides of [SEQ ID NO:15] <u>SEQ ID NO:1</u>

- 188. The probe of claim 182 or 183 comprising DNA.
- 189. The probe of claim 182 or 183 comprising a peptide nucleic acid.
- 190. The probe of claim 182 or 183 further comprising a detectable label.
- 191. The probe of claim 190 wherein the detectable label is a fluorescent label.
- 192. A method comprising:
- (a) providing a test sample comprising nucleic acid molecules present in a biological sample obtained from an individual;
- (b) contacting the test sample with a probe comprising at least 15 contiguous nucleotides of the nucleotide sequence of [SEQ ID NO:15] <u>SEQ ID NO:1</u>, the probe comprising at least one of:
  - (i) nucleotide 120 of SEQ ID NO:1 wherein T is replaced by C;
  - [i] (ii) nucleotide 464 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u> is replaced by G;
  - [ii] (iii) nucleotide 519 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>C</u> is replaced by T;
  - [iii](iv) nucleotide 668 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N] C is
  - replaced by T;
    - [iv] (v) nucleotide 1059 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u>
  - is replaced by C;
  - [v] (vi) nucleotide 1289 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>C</u>
  - is replaced by A;
  - [vi] (vii) nucleotide 1308 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N]  $\underline{T}$  is replaced by C; and

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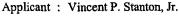
[vii] (viii) nucleotide 1784 of [SEQ ID NO:15] SEQ ID NO:1 wherein [N] G

is replaced by A;

or the complement thereof; and

- (c) determining if the test sample comprises a nucleic acid molecule that hybridizes to the probe.
  - 193. A method comprising:
- (a) providing a test sample comprising nucleic acid molecules present in a biological sample obtained from an individual;
- (b) contacting the test sample with a probe comprising at least 15 contiguous nucleotides of the nucleotide sequence of [SEQ ID NO:15] <u>SEQ ID NO:1</u>, the probe comprising at least two of:
  - (i) nucleotide 120 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u> is replaced by C;
  - (ii) nucleotide 464 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N]  $\underline{T}$  is replaced by G;
  - (iii) nucleotide 519 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N]  $\underline{C}$  is replaced by T;
  - (iv) nucleotide 668 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N]  $\underline{C}$  is replaced by T;
  - (v) nucleotide 1059 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>T</u> is replaced by C;
  - (vi) nucleotide 1289 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>C</u> is replaced by A;
  - (vii) nucleotide 1308 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N]  $\underline{T}$  is replaced by C; and
  - (viii) nucleotide 1784 of [SEQ ID NO:15] <u>SEQ ID NO:1</u> wherein [N] <u>G</u> is replaced by A;

or the complement thereof; and



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probe.

(c) determining if the test sample comprises a nucleic acid molecule that hybridizes to the

- 194. The method of claim 192 or 193 wherein the probe comprises no more than 500 contiguous nucleotides of [SEQ ID NO:15] SEQ ID NO:1.
- The method of claim 192 or 193 wherein the probe comprises no more than 200 contiguous nucleotides of [SEQ ID NO:15] SEQ ID NO:1.
- 196. The method of claim 192 or 193 wherein the probe comprises no more than 100 contiguous nucleotides of [SEQ ID NO:15] SEQ ID NO:1.
- The method of claim 192 or 193 wherein the probe comprises no more than 50 197. contiguous nucleotides of [SEQ ID NO:15] SEQ ID NO:1.
  - 198. The method of claim 192 or 193 wherein the probe is a DNA probe.
- 199. The method of claim 192 or 193 wherein the probe is a peptide nucleic acid probe.
  - 200. The method of claim 192 or 193 wherein the probe comprises a detectable label.
  - 201. The method of claim 200 wherein the detectable label is a fluorescent label.